Investigating Low Salinity Immersion as a Potential Mitigation Strategy for MSX in Eastern Oysters

INTRODUCTION

Multinucleate Sphere Unknown (MSX) is an oyster disease caused by the pathogenic parasite *Haplosporidium nelsoni*. MSX can infect Eastern oysters (*Crassostrea virginica*) of all ages, from spat to adults, causing up to 95% mortality rates (Haskin & Ford, 1979; Stephen & McGladdery, 2003). Water temperature and salinity are environmental factors influencing MSX severity (i.e., infection rates and associated mortality). MSX infections are acquired at water temperatures between 5 and 20°C (Ford, 1985). Peak mortalities of susceptible oysters occur when water temperatures return below 20°C after high summer temperatures, although mortality can vary from five weeks to ten months after infection depending on prevalence, temperature, and salinity (Andrews, 1966; Haskin & Andrews, 1988). Previous studies have investigated the effects of low salinity on *H. nelsoni*; the findings of four significant papers are summarized below:

1. Sprague et al. (1969) held infected oysters at three salinities (7-8 ppt, 14-16 ppt, 19-22 ppt) for six months. This study found that MSX prevalence was highly influenced by salinity, showing final prevalences (by histology) of 6% (7-8 ppt), 63% (14-16 ppt), and 89% (19-22 ppt).
2. Andrews (1983) found that *H. nelsoni* cells were still present in histological sections of oysters collected in the James River at the beginning of May, even though salinities had been <10 ppt. However, they were no longer detectable by mid-May after water temperatures increased. This study suggested that the correlation of parasite loss with increased temperature and heightened oyster activity may indicate that *H. nelsoni* cells were actively expelled by the host defense reactions under low salinity.
3. Ford (1985) moved infected oysters from a high salinity area (>20 ppt) to a low salinity area (<10 ppt). After one week, *H. nelsoni* cells appeared to be in poor condition. After two weeks, *H. nelsoni* cells were no longer detected histologically. This study concluded that parasites were probably severely damaged within a day or two of moving to low salinity water and, when sampled after one week, the parasites were dead or moribund.
4. Ford and Haskin (1988) compared salinity tolerance of *H. nelsoni* to that of oyster hemocytes to determine salinity-tolerance thresholds. Parasite destruction began at salinities <15 ppt and increased exponentially at salinities <9 ppt, at which point maximum damage had occurred due to loss of cell membrane integrity. This study concluded that reduced incidence of *H. nelsoni* in low salinity was likely due to a physiological inability to tolerate reduced salinity, rather than enhanced effectiveness of host defense mechanisms. This indicated that parasites seen in histological sections in Andrews (1983) in early May were likely already dead, but preserved by low temperatures. Increasing temperature may therefore speed degradation of parasites and removal by phagocytes, resulting in their disappearance from histological sections.

In the United States, where many oyster growing areas are within river systems with significant salinity gradients, low salinity immersion has been suggested as a management strategy. ICES (2010) suggested maintaining oysters in low salinity waters as long as possible and, if final conditioning at higher salinity is needed for market, then conditioning should be done late in the season to avoid the major early-summer infection period. They additionally suggest that immersing oysters in waters <10 ppt for two to three weeks above 20°C may eliminate the parasite from infected oysters. The majority of PEI oyster growing and harvesting occurs in high salinity waters (typically >20 ppt) therefore moving significant quantities of

oysters to naturally low salinity areas throughout the early-summer infection period is likely not a practical management strategy. This study was therefore conducted to investigate if low salinity immersion treatments could be used as a potential MSX mitigation strategy for Eastern oysters in PEI.

This study was divided into two phases. Phase 1 consisted of a preliminary trial conducted in January 2025. The objective of Phase 1 was to conduct a small-scale trial to determine if PEI oysters had a positive response to low salinity immersion and, if so, to investigate an optimal treatment duration. Phase 2 incorporated a field component and a larger sample size to evaluate the following objectives:

1. To investigate treatment durations of 7 and 11 days based on the results from Phase 1;
2. To investigate the timing and frequency of treatments (July, October, both);
3. To compare two year classes (2023 and 2024);
4. To track mortality for several months following the treatments and compare with oysters that did not undergo a low salinity treatment, to inform if there are major benefits to low salinity immersion.

METHODS

Phase 1. January 2025

Live oysters (n = 270) were collected from a lease site in Lennox Channel that had been pre-determined to have high MSX prevalence. Upon returning to the PEI Aquaculture Division's warehouse facility in Charlottetown, 20 oysters were measured (58 to 80 mm in length, average 68 mm) and tested by qPCR to obtain an initial infection level. The remaining oysters were divided into three treatment groups: 1) freshwater (0 ppt) using tap water, 2) low salinity (8-10 ppt) using tap water mixed with ambient seawater, and 3) high salinity (28-31 ppt) using ambient seawater (Figure 1).

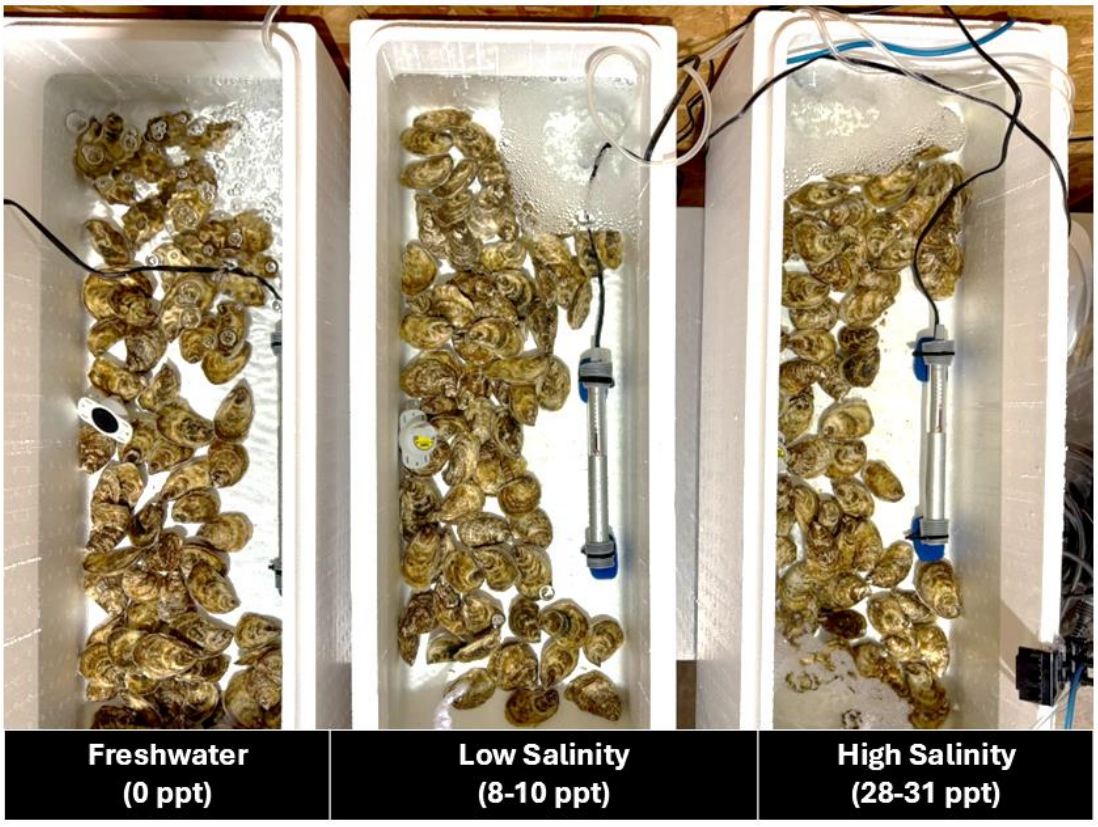

Figure 1. Phase 1 set up with three treatment groups: freshwater, low salinity, and high salinity.

All oysters were gradually acclimatized from a water temperature of -0.7°C to 20°C over a 24h period. The water was maintained between 20 and 21°C for the duration of the trial using Top Fin Submersible Aquarium Heaters. Water temperature was additionally tracked using an Onset HOBO TidbiT MX2203 Wireless Temperature Data Logger (Figure 2). Each treatment group was held in 40L of water, ranging from 0.48L/oyster at the start of the trial to 1.33L/oyster by the end of the trial. The water was aerated using a Top Fin Aquarium Air Pump. Dissolved oxygen, water temperature, and salinity were recorded intermittently using a YSI Pro2030 Dissolved Oxygen and Conductivity Meter. The water in each treatment was replaced every 2-4 days to prevent waste build-up.

Figure 2. Water temperature monitored using an Onset HOBO Tidbit MX2203 logger.

Ten oysters were removed from each treatment on days 2, 4, 7, 11, and 14 for diagnostic testing. Moribund oysters were additionally removed and tested throughout the trial. On day 15, approximately 30 oysters remained in each treatment group, and all water was replaced by ambient seawater (30 ppt) to observe the effects of re-introduction to high salinity. The trial was terminated on day 18 when all remaining oysters were removed for diagnostic testing.

Diagnostic testing was conducted by taking two cross-sections, 1.0 to 2.5 inches posterior to the hinge, ensuring coverage of the gills, mantle, and digestive tract. One cross-section was used to determine MSX prevalence by qPCR, while the other was used for histological analysis. Histological examination was conducted using light microscopy of paraffin embedded tissue sections. Prevalences of *H. nelsoni* infection and sporulation were quantified, as well as infection severity scored as 0 = none, 1 = mild, 2 = moderate, and 3 = advanced.

Phase 2.1. July 2025

In mid-July 2025, live oysters (n = 5,250 from 2023 year class, 8,400 from 2024 year class) were collected from lease sites in Lennox Channel that had been pre-determined to have high MSX prevalence. Thirty oysters were measured from each year class. The oysters were immediately sorted and placed in vexar bags (n = 42) according to their respective groups (Figure 3) at densities of 250 oysters/bag and 400 oysters/bag for the 2023 and 2024 year classes, respectively. Each group consisted of three bags for triplicate analysis.

2023 Year Class (250 oysters/bag x 3 bags/group x 7 groups = 5,250 oysters)

Control group, no low salinity treatment	July treatment, 7 day exposure time.	July treatment, 11 day exposure time.	October treatment, 7 day exposure time.	October treatment, 11 day exposure time.	Both treatments, 7 day exposure time.	Both treatments, 11 day exposure time.

2024 Year Class (400 oysters/bag x 3 bags/group x 7 groups = 8,400 oysters)

Control group, no low salinity treatment	July treatment, 7 day exposure time.	July treatment, 11 day exposure time.	October treatment, 7 day exposure time.	October treatment, 11 day exposure time.	Both treatments, 7 day exposure time.	Both treatments, 11 day exposure time.

Figure 3. Trial set up with the oysters sorted into seven groups within each year class.

The control and October treatment groups were immediately returned to the field site in off-bottom cages (6 bags/cage). Hourly water temperature and salinity was recorded at bag height (<1 m below surface) using a Star-Oddi DST CTD logger. The "July treatment" and "both treatment" groups were transported to the PEI Aquaculture Division's warehouse facility in Charlottetown for low salinity immersion (Figure 4). The treatment water was maintained around 21°C and 9 ppt, created by mixing freshwater with Instant Ocean solution. Each tank contained approximately 700L of water. At the beginning of the treatment, there were 3,000 oysters in the tank containing the 2023 year class and 4,800 oysters in the tank containing the 2024 year class. However, high mortality rates (see results) caused a decrease in the number of oysters in each tank throughout the treatment.

Figure 4. Phase 2 low salinity treatment set-up with 2024 year class (left) and 2023 year class (right).

Water temperature was recorded hourly using an Onset HOBO TidbiT MX2203 Wireless Temperature Data Logger. The water was aerated using Top Fin Aquarium Air Pumps and 264 GPH Statuary Fountain Pumps, to maintain dissolved oxygen levels around 70%. Dissolved oxygen was recorded hourly using an aquaMeasure DO Wireless Sensor. Additionally, dissolved oxygen, water temperature, and salinity were recorded intermittently using a YSI Pro2030 Dissolved Oxygen and Conductivity Meter. Every 2-3 days,

dead oysters were removed and the live oysters were placed in new water, rotating the position of the bags in the tanks to limit stacking effects.

MSX infection levels were determined using histological analysis only (n = 15 oysters), foregoing qPCR based on inconclusive results during Phase 1. Oysters from representative groups were tested before and after the low salinity treatment. All surviving oysters were returned to the field site in Lennox Channel immediately after treatment (day 7 or 11). Mortality was subsequently assessed in August and September.

Phase 2.2. October 2025

In early October 2025, the "October treatment" and "both treatments" groups were retrieved from the field site in Lennox Channel and transported to the Aquaculture Division's warehouse facility in Charlottetown for low salinity immersion. Water conditions in the tanks were consistent with the July treatment (21°C, 9 ppt). For the October treatment, oysters were held in trays (rather than bags, as used in the July treatment) with the goal to improve water quality (Figure 5). Due to significant mortality since the beginning of the study, the number of oysters in each tank was lower than during the July treatment. At the start of the October treatment, there were approximately 1700 oysters in the tank containing the 2023 year class and approximately 2800 oysters in the tank containing the 2024 year class. Again, high mortality rates (see results) caused a decrease in the number of oysters in each tank throughout the treatment.

Figure 5. Oysters exposed to low salinity treatment in vexar bags (July) and trays (October).

Based on diagnostic results from Phase 2.1, MSX infection levels were determined using both qPCR and histological analysis (n = 15 oysters). Oysters were tested before and after the low salinity treatment, then again in late October, two weeks after all surviving oysters had been returned to the field site. All groups were assessed for mortality and measured for growth. The oysters (bagged and held in cages) were subsequently sunk for the winter. Mortality will be reassessed when the oyster cages are raised in spring 2026.

RESULTS

Phase 1. January 2025

Oyster Mortality

Negligible mortality was observed (3.6%, 9/250) throughout the 18 day trial period. Of the 9 oysters that died, 5 oysters were in the freshwater treatment, 1 oyster was in the low salinity treatment, and 3 oysters were in the high salinity treatment.

MSX Prevalence by qPCR

Figure 6 details MSX prevalence by qPCR throughout the trial period. MSX prevalence was 85% (n = 20) before the trial began. Prevalence varied from 30% to 100% (n = 10) throughout the 14-day treatment period, with no consistent patterns between treatments. On day 18, MSX was detected >90% (n = 30) for all groups following three days of high salinity immersion.

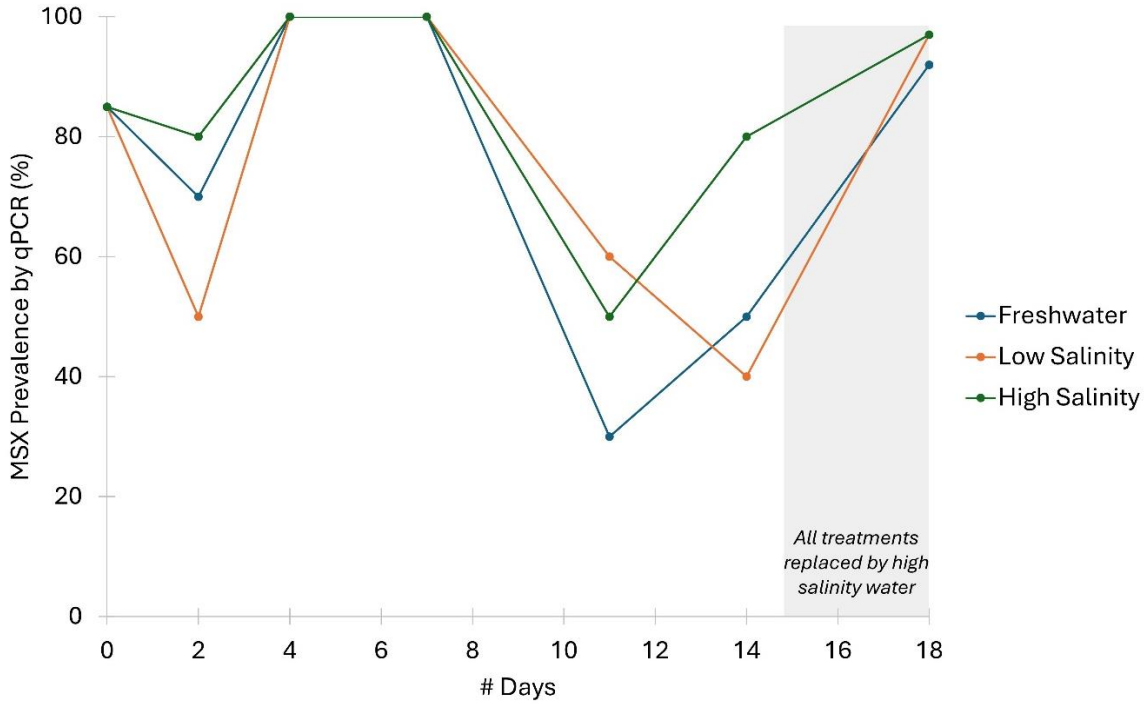

Figure 6. MSX prevalence by qPCR during Phase 1. 20 oysters were tested on day 0. 10 oysters were tested from each treatment on days 2 to 14. The remainder of the oysters (approximately 30) were tested from each treatment on day 18.

MSX Prevalence and Viability by Histology

Histological analysis was conducted on a subset of samples to collect information on MSX prevalence, infection severity, and plasmodia viability. Oysters collected on days 0, 7, and 14 were analysed for all three treatment groups, in addition to days 11 and 18 for the low salinity treatment only (Table 1). Many of the tissues collected on day 0 were unable to be fully analysed by histology due to issues with preservation, therefore day 0 prevalence by histology is not included here, as it would be misrepresented. The tissues

analysed throughout the remainder of the treatment were adequately preserved and showed low detections of *H. nelsoni* by histology.

Table 1. Number of oysters with detections of *H. nelsoni* by histology.

Day	Freshwater Treatment	Low Salinity Treatment	High Salinity Treatment
0	Inadequate condition for analysis.		
7	1/10	1/10	1/10
11	NA	1/10	NA
14	0/10	0/10	1/10
18	NA	6/33	NA

Visual observations suggested a shift in the viability of *H. nelsoni* plasmodia throughout the low salinity treatment (Figure 7). Normal, viable plasmodial stages of *H. nelsoni* were observed on day 0. Day 7 contained examples of enlarged plasmodial stages with enlarged nuclei, possibly indicating parasite bloating. Day 11 contained examples of degraded plasmodial stages of *H. nelsoni*.

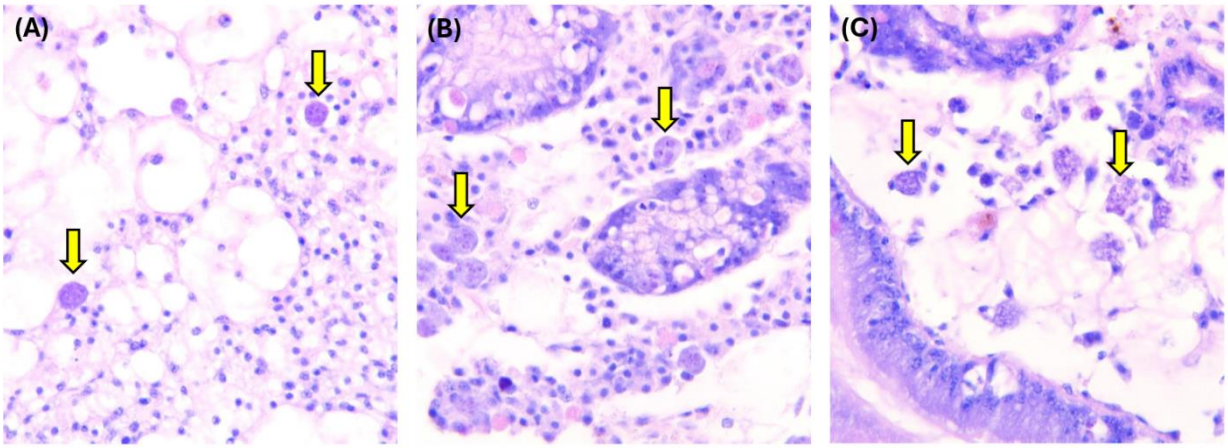

Figure 7. A) Example of normal, viable plasmodial stages of *H. nelsoni* on day 0. B) Example of enlarged plasmodial stages on day 7. C) Example of degraded plasmodial stages on day 11.

Phase 2. July to October 2025

Environmental Conditions

At the field site in Lennox Channel, water temperature was 25-26°C when the oysters were first collected to begin the trial in mid-July. Water temperature gradually decreased to 13-14°C by early October when the second treatment began (Figure 8). Salinity showed very little fluctuation, maintaining around 28 ppt. The steep dips in salinity in Figure 8 indicate when the cages were flipped up for air drying. Water temperature and salinity are continuing to be recorded, and the remainder of the data will be retrieved in spring 2026.

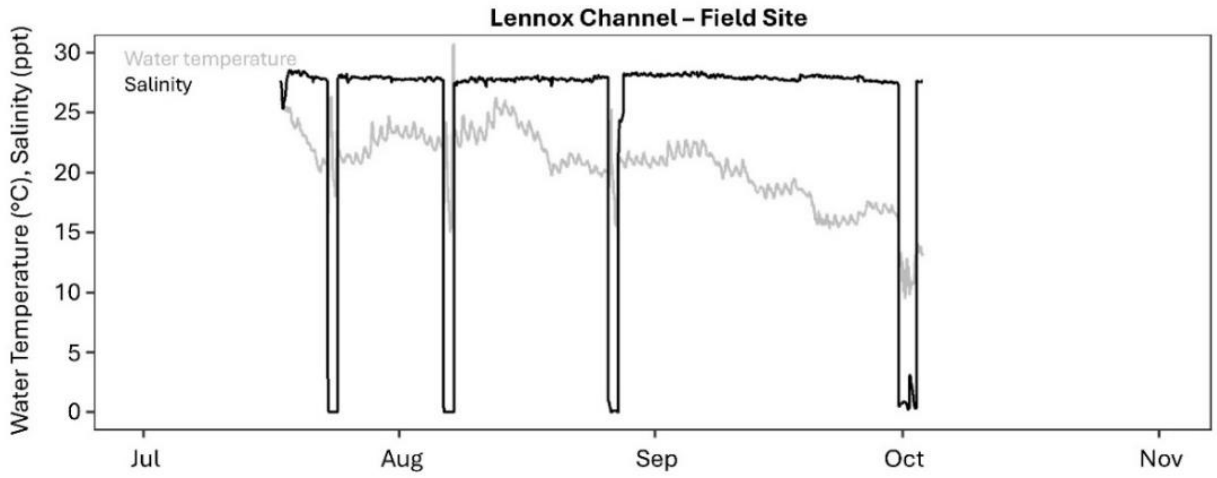

Figure 8. Water temperature and salinity at the field site in Lennox Channel. The steep dips in salinity indicate when the cages were flipped up for air drying.

Oyster Mortality

By the end of October, cumulative mortality ranged from 60-90% in the groups that experienced one or both low salinity treatments, compared to 35% (2024 year class) and 50% (2023 year class) in the control groups (Figure 9). During the July treatment, cumulative mortality was greater for the 2023 year class (>50%) compared to the 2024 year class (approx. 25%). This may be the result of a pump failure in the tank containing the 2023 year class, which resulted in dissolved oxygen levels between 20 and 50% for 36h, compared to 70% at which it was regularly maintained throughout the treatments. However, mortality rates were similar during the October treatment when oxygen levels were maintained at 70%, therefore indicating that low oxygen levels were not the sole contributing factor to high mortality. Cumulative mortality will be reassessed in spring 2026.

Oyster Growth

At the beginning of the trial, the 2023 year class ranged from 34 to 66 mm (average 51 mm) in length and the 2024 year class ranged from 20 to 41 mm (average 32 mm) in length. By late October, the 2023 year class showed -1.7 to -6.9 mm growth, possibly indicating that there was greater mortality in the larger oysters within that age class (Table 2). The 2024 year class showed 1.3 to 3.2 mm growth, which is much less than would typically be observed for this age class. Notably, many industry members in western PEI indicated low growth in 2025 across all age groups, except 2025 seed. This may possibly be attributed to low rainfall, and thereby low food availability, throughout the season, and/or MSX infection pressure.

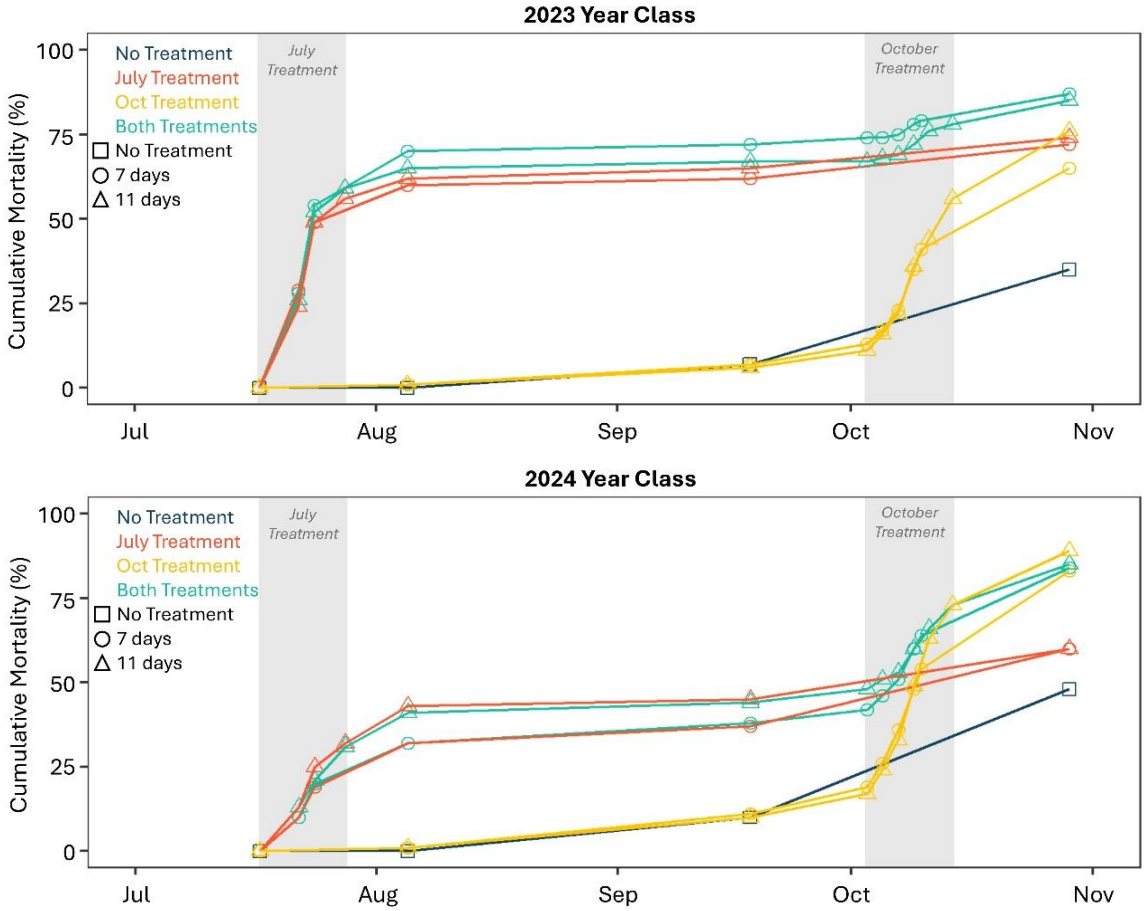

Figure 9. Cumulative mortality throughout Phase 2 of the low salinity trials. The grey boxes indicate the timing of the July and October treatments.

Table 2. Average length (mm) of oysters measured in July and October 2025.

	2023 Year Class			2024 Year Class		
	Jul 17th	Oct 29th	Growth	Jul 17th	Oct 29th	Growth
No treatment	51.4	49.8	-1.7	31.6	34.1	2.6
July treatment, 7 days		47.2	-4.2		33.7	2.1
July treatment, 11 days		45.2	-6.3		32.8	1.3
October treatment, 7 days		47.4	-4.0		34.4	2.8
October treatment, 11 days		47.2	-4.2		33.2	1.7
Both treatments, 7 days		45.0	-6.5		33.4	1.9
Both treatments, 11 days		44.6	-6.9		34.7	3.2

MSX Infection Dynamics by qPCR and Histology

Diagnostic testing by qPCR was not conducted during the July treatment due to the inconclusive results obtained during Phase 1. Histology was instead prioritized as a more informative diagnostic method given that the population was assumed to be heavily infected. However, only 1/30 oysters had detections of *H.*

nelsoni by histology before the treatment, and 0/58 after the treatment. Therefore, both qPCR and histology were conducted for the October treatment.

All oysters tested positive by qPCR in October (before, after, and two-week post treatment). The cycle threshold (Ct) values (Figure 10) suggest that the most severe infections were observed before the treatment (lower Ct values), and the least severe infections were observed two-weeks post treatment (highest Ct values).

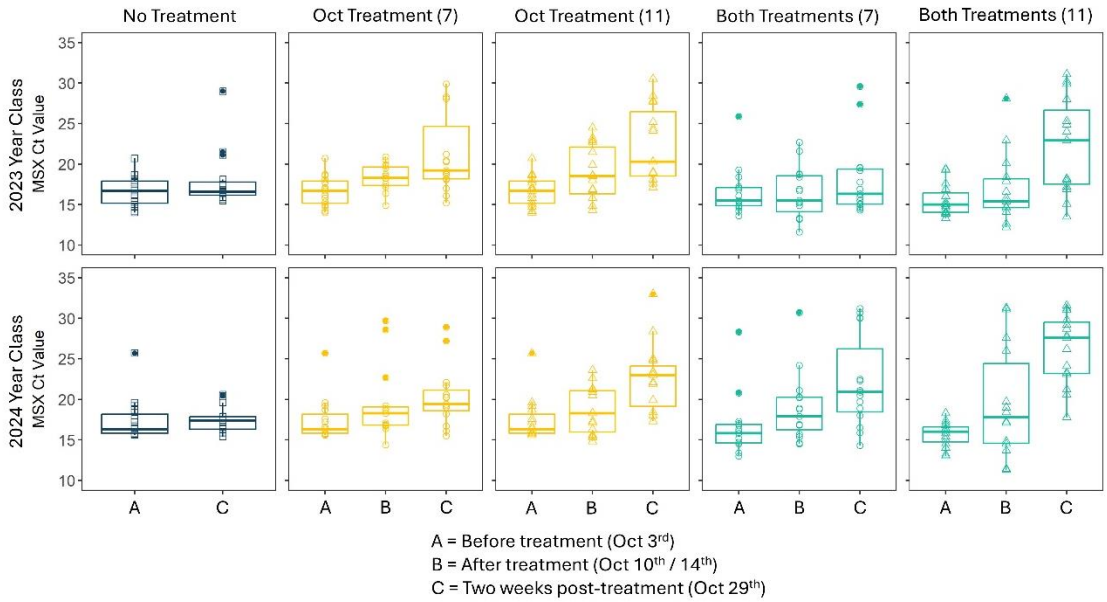

Figure 10. MSX Ct values based on qPCR testing A) directly before the October treatment, B) directly after the October treatment, and C) two weeks after the oysters had been returned to the field site.

Upon comparing all groups on October 29th (Figure 11), the groups treated in October had presumably less severe infections (higher Ct values) than those treated in July or not treated at all. Additionally, the 11 day treatments had higher Ct values than the 7 day treatments.

Histological analysis (Figure 12) indicated that total MSX infection remained similar directly before and after the October treatment (90-100%), except for one group (2024 year class, both treatments, 11 days) which decreased to 71%. However, the total infection prevalence dropped to 50-90%, two weeks after returning to the field site, for all groups treated in October. This may indicate the poor quality of some of the plasmodia observed directly after the treatment, which may have been degraded and removed once the oysters were returned to the field site. The prevalence of advanced MSX infections was high (87-93%) before the treatment and showed a considerable decrease directly after the treatment (33-87%), and a further decrease (10-87%) two-weeks post treatment. Oysters that had been treated for 11 days generally showed lower prevalences of advanced infections than those treated for 7 days. A similar trend was reflected in the average MSX severity score. MSX plasmodia viability was scored, showing a decrease in viability from a score of 1 (fully viable) before the treatment to scores ranging from 0.5-0.8 after the treatment.

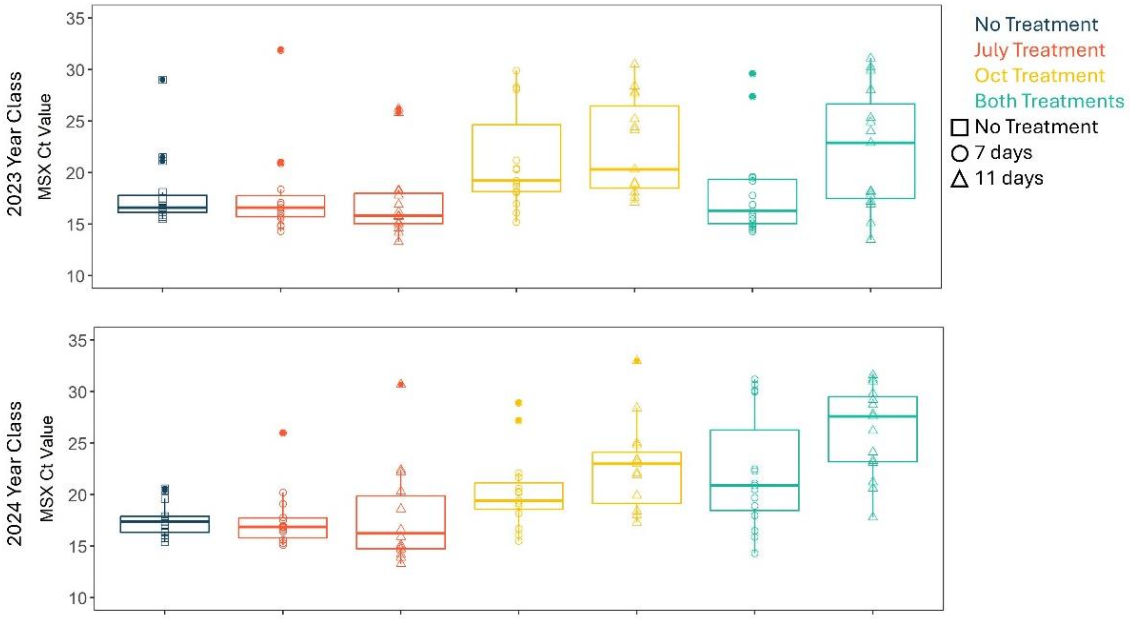

Figure 11. MSX Ct values based on qPCR testing comparing all groups, tested on October 29th, 2025.

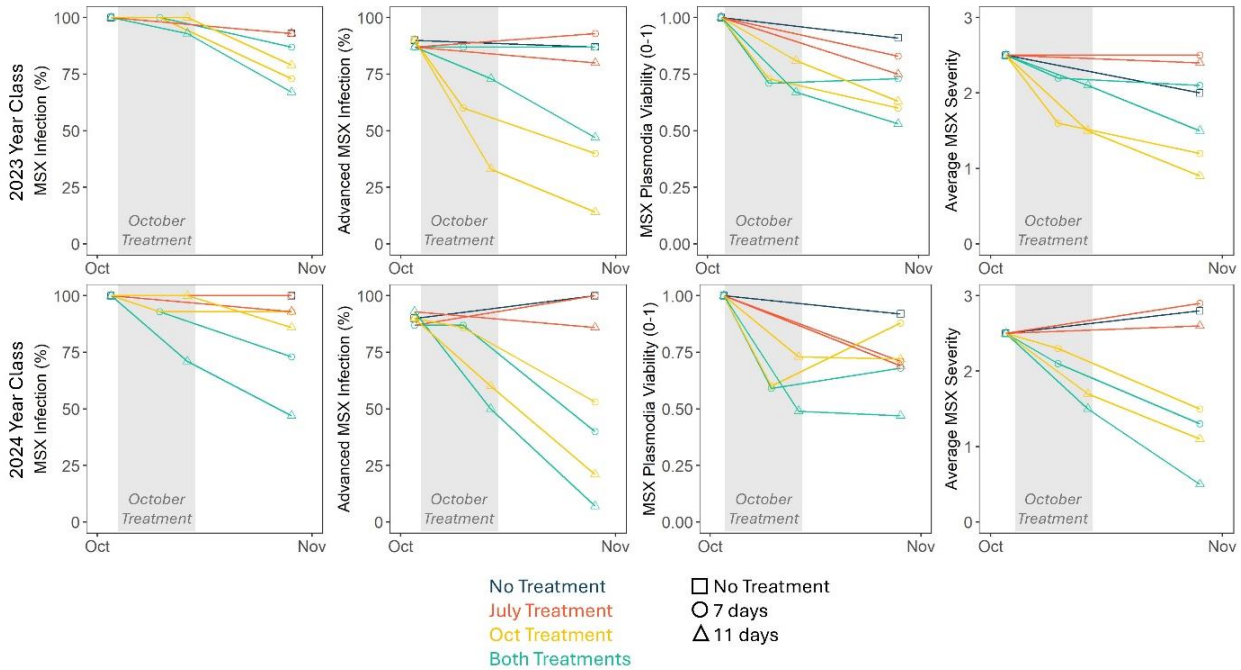

Figure 12. Histological results directly before and after the October treatment, and two weeks after the oysters had been returned to the field site.

NEXT STEPS

All treatment groups will be re-assessed in spring 2026. Next steps will be determined based on those results.

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